Volume 214, number 1, 62–64 FEB 04549 April 1987

Differential effects of cadmium on cytosolic and mitochondrial glutathione levels in the rat heart

Ijaz S. Jamall

Toxicology Program, St. John's University, Jamaica, NY 11439, USA

Received 27 January 1987

In rats fed a low-selenium diet, cytosolic GSH levels in the heart were significantly elevated when compared to rats fed a selenium-supplemented diet. Similar results were obtained in cardiac cytosol from rats treated with either parenteral or dietary Cd. Mitochondrial GSH was not affected by cadmium in selenium-deficient rats, However, significant elevations of mitochondrial GSH were seen in cadmium-treated rats fed either 0.1 or 0.5 ppm selenium. The alterations in cytosolic GSH levels correlate with reductions in GSH-Px activity reported earlier [(1987) Toxicol. Appl. Pharmacol. 87, in press]. However, levels of mitochondrial GSH did not correlate with GSH-Px activity.

Glutathione; Cadmium; Selenium; Cytosol; Mitochondria; (Heart)

1. INTRODUCTION

The reduced form of the tripeptide, glutathione (GSH), is depleted in the hypoxic heart [1,2]. Depressed cardiac contractility, following reperfusion injury in the isolated rabbit heart, has also been shown to involve significant reductions in myocardial GSH with no change in oxidized glutathione, glutathione reductase or glutathione peroxidase (GSH-Px) activities [3].

Work in our laboratory has demonstrated that cadmium cardiotoxicity is mediated by increased lipid peroxidation [4,5]. This peroxidative injury occurs only when the activity of the selenoenzyme, GSH-Px, is markedly reduced in cardiac cytosol and mitochondria [6]. These Cd-induced reductions in GSH-Px activity were attributed to Cd-Se interactions in the heart since increasing dietary Se resulted in increased accumulations of Cd and Se in these hearts [6]. The existence of Cd-Se complexes has been demonstrated in erythrocytes [7].

Since excess GSH is added to the incubation mixture used to assay the activity of the selenoenzyme, GSH-Px [8], and since Cd has been shown to complex with GSH in vitro [9], it is entirely plausible that the Cd-induced reductions in cytosolic and mitochondrial GSH-Px activity in the heart [6] could be due to Cd-induced depletion of GSH rather than via Cd-Se interactions. Such Cd-GSH interactions could also contribute to the increased lipid peroxidation seen in the heart of these rats.

To elucidate the mechanism for the Cd-induced reductions in GSH-Px, we determined GSH levels in cytosol and mitochondria from rats treated as described [6].

Correspondence address: I.S. Jamall, Toxicology Program, St. John's University, Jamaica, NY 11439, USA

2. MATERIALS AND METHODS

2.1. *Biochemicals*

Reduced glutathione (GSH) and *o*-phthalaldehyde (OPT) were obtained from Sigma. All other chemicals were of reagent grade and obtained from Fisher.

2.2. *Animal treatment*

54 male weanling Sprague-Dawley rats (Taconic Farms, Germantown, NY) were randomly assigned to the various treatment groups. The rats were fed

Published by Elsevier Science Publishers B.V. (Biomedical Division)

a Torula yeast-based feed low in Se (less than 0.02 ppm by analysis, from Dyets, PA) and were given deionized drinking water supplemented with 0, 0.1 or 0.5 ppm Se, as sodium selenite. The ingredients of the basal feed have been described [4,6]. There were two groups of Cd-treated rats. The first group received the basal diet supplemented with 0, 0.1 and 0.5 ppm Se for 5 weeks. At the end of the 5th week, these rats were given 5 mg Cd (as $CdCl_2$) via osmotic minipumps (Alzet 2002) implanted subcutaneously in the dorsal neck region under light pentobarbital anesthesia. The second group of Cd-treated rats received 50 ppm Cd (as $CdCl_2$) admixed with their feed for the entire 7-week experimental period.

2.3. *Necropsy and tissue homogenization*

At the end of 7 weeks, all animals were fasted for 12–16 h and killed by decapitation. The heart was excised, weighed, blotted dry and minced using stainless-steel scissors. A 10% (w/v) homogenate in 0.25 M sucrose was prepared using a motor-driven Teflon pestle. Mitochondrial and cytosolic fractions were obtained by differential centrifugation [10].

2.4. *Assay for GSH in heart cytosol and mitochondria*

GSH was determined in cytosolic and mitochondrial fractions within 3 h of necropsy by the fluorometric method of Hissin and Hilf [11]. Reduced GSH (Sigma) was used to construct the standard curve. Hence all values are reported as GSH equivalents, normalized for protein in the sample. Protein was determined according to the dye-binding method in [12].

2.5. *Statistical analysis*

The data obtained were analyzed by the two-way analysis of variance (ANOVA) for possible significant interactions between the various treatment groups. Where interactions were found to be significant ($P < 0.05$) by the two-way ANOVA, significance among particular groups was determined using the Fisher's least significant difference (LSD) procedure. Since the objective of this study was to compare the effects of Cd at various levels of dietary Se, comparisons by the LSD test were made between either of the two Cd treatments and rats fed identical levels of Se without any Cd treatment.

3. RESULTS

Rats fed the basal, low-Se diet (0 ppm Se added) exhibited a 50% increase in cytosolic GSH compared to rats fed the diet supplemented with 0.1 or 0.5 ppm Se without Cd (−Cd). Treatment with

Table 1

Effects of Cd on cytosolic and mitochondrial GSH in heart of rats fed 50 ppm Cu and various levels of Se for 7 weeks

	Dietary Se (ppm)	− Cd	+ Cd-osmotic pumps	Cd-diet
Cytosol				
GSH	0	18.6 ± 1.25	24.6 ± 0.574[a]	22.3 ± 1.65[a]
(nmol/mg protein)	0.1	12.4 ± 0.602	18.9 ± 2.31[b]	18.7 ± 1.85[b]
	0.5	11.2 ± 1.74	13.5 ± 0.977	17.3 ± 2.12[b]
Mitochondria				
GSH	0	11.3 ± 0.816	11.1 ± 0.313	11.4 ± 1.93
(nmol/mg protein)	0.1	8.72 ± 1.59	11.1 ± 2.93[a]	12.7 ± 0.671[a]
	0.5	7.96 ± 1.54	11.2 ± 0.565[a]	11.3 ± 0.593[a]

All values are reported as the $x \pm$ SD of 6 rats per group. Statistical significance was calculated by the two-way ANOVA. Significance for Cd effects was determined between − Cd and either + Cd-osmotic pump or + Cd-diet groups by the LSD test and indicated as: [a] $P < 0.05$, [b] $P < 0.01$

parenteral or dietary Cd resulted in 20–50% elevations of cytosolic GSH at all levels of dietary Se.

As observed in the cytosol, Se-deficient rats exhibited a 30% higher mitochondrial GSH compared to rats fed the Se-supplemented diets in the absence of Cd treatment (table 1). Parenteral or dietary Cd did not significantly affect mitochondrial GSH levels in rats fed the basal low-Se diet. However, rats fed the 0.1 or 0.5 ppm Se diets and given parenteral or dietary with Cd showed an approx. 38% elevation in mean mitochondrial GSH levels.

4. DISCUSSION

Treatment of rats with 5 mg Cd via osmotic pumps or feeding them 50 ppm Cd for 7 weeks does not deplete cytosolic or mitochondrial GSH in the heart. The levels of GSH reported in this paper are similar to values reported for the rabbit myocardium [3]. The elevated GSH levels in cytosolic fractions of the Se-deficient rat heart, or rats treated with Cd, exhibit a negative correlation ($r = -0.88936$) with the reduction in the activity of the cytosolic GSH-Px in these animals [6]. This suggests that the elevation in cytosolic GSH is a consequence of reductions in GSH-Px activity in this compartment, by dietary Se deficiency and/or by Cd treatment. Similar observations were made with respect to GSH levels in cardiac mitochondria from Se-deficient and Se-supplemented rats, not treated with Cd (table 1). However, the elevated levels of mitochondrial GSH in Cd-treated rats cannot be explained by Cd-induced reductions in GSH-Px activity since dietary Cd did not affect mitochondrial GSH-Px activity [6]. No significant correlation was observed between mitochondrial GSH-Px activity [6] and GSH levels ($r = -0.17643$).

Isolated hepatocytes contain two distinct pools of GSH, a cytoplasmic pool and a mitochondrial one, with the latter pool being more resistant to GSH depletion in vitro [13]. A similar compartmentation of GSH in the heart might explain the differential effects of Cd in the mitochondrial fraction relative to the cytosolic one. Since dietary Cd does not affect mitochondrial GSH-Px activity [6] yet affects elevations in GSH levels in this compartment (table 1), Cd might affect mitochondrial GSH synthesis, or transport into this compartment.

Whatever the precise mechanism for the effects of Cd on mitochondrial GSH, it is clear from our earlier study [6] and the present data that GSH depletion is not involved in Cd-induced peroxidative injury seen in the heart of rats fed 0 or 0.1 ppm Se for 7 weeks. Rather, such Cd-induced peroxidative injury is secondary to Cd-induced reductions in cytoplasmic and mitochondrial GSH-Px activities. Studies are currently in progress to elucidate the mechanism of Cd effects on cardiac mitochondria.

ACKNOWLEDGEMENTS

This work was funded by NIH grant no. ES-03370. I thank Thomas W. Simmons for his helpful discussions.

REFERENCES

[1] Guarnieri, C., Flamigni, F. and Rossoni-Calderara, C. (1979) Biochem. Biophys. Res. Commun. 89, 678.
[2] Guarnieri, C., Flamigni, F. and Calarera, C.M. (1980) J. Mol. Cell. Cardiol. 12, 797.
[3] Curello, S., Ceconi, C., Bigoli, C., Ferrari, R., Albertini, A. and Guarnieri, C. (1985) Experientia 41, 42–43.
[4] Jamall, I.S. and Smith, J.C. (1985) Toxicol. Appl. Pharmacol. 80, 33–42.
[5] Jamall, I.S. and Smith, J.C. (1986) in: Handbook of Experimental Pharmacology (Foulkes, E.C. ed.) vol.80, pp.351–361, Springer, Berlin.
[6] Jamall, I.S. and Sprowls, J.J. (1987) Toxicol. Appl. Pharmacol. 87, in press.
[7] Gasiewicz, T.A. and Smith, J.C. (1978) Environ. Health Perspect. 25, 133–136.
[8] Burk, R.F., Lawrence, R.A. and Lane, J.M. (1980) J. Clin. Invest. 65, 1024–1031.
[9] Perrin, D.D. and Watt, A.E. (1971) Biochim. Biophys. Acta 230, 96–104.
[10] Murfitt, R.R., Stiles, J.W., Powell, W.J. jr and Sanadi, R. (1978) J. Mol. Cell. Cardiol. 10, 109–123.
[11] Hissin, P.J. and Hilf, R. (1976) Anal. Biochem. 74, 214–226.
[12] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[13] Meredith, M.J. and Reed, D.J. (1982) J. Biol. Chem. 257, 3747–3753.